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New and Improved Vaccines Against Meningococcal Disease

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I. BACKGROUND

A. Causative Agent

Meningococcal disease in its various forms is caused by *Neisseria meningitidis*, an aerobic, gram-negative diplococcus. Its sole natural habitat is human mucosal membranes, primarily the nasopharynx, which it normally colonizes without causing disease. Virulent strains freshly isolated from the blood or cerebrospinal fluid are typically encapsulated, whereas throat isolates may or may not be encapsulated. The capsules are composed of anionic polysaccharides, which are the basis for classification of the species into serogroups. Twelve different serogroups are current recognized: A, B, C, 29E, H, I, K, L, W135, X, Y, and Z. The respective capsular polysaccharides (CP) have all been chemically and structurally defined [1].

Meningococci have been further subdivided into serotypes, serosubtypes, and immunotypes on the basis of the antigenic specificity of two major outer membrane proteins (OMP) and the lipopolysaccharide (LPS) [2]. The serotype and serosubtype are based on antigenic variation of the porins PorB and PorA respectively and are defined on the basis of reactivity with specific monoclonal antibodies. About 18 different serotype determinants, 13 serosubtype determinants, and 12 LPS immunotypes have been identified. These subcapsular antigens are independent of the serogroup. A recommended nomenclature for specifying the major antigens on a given strain has been published [2]. According to this nomenclature, a strain is described by a strain number followed parenthetically by serogroup: serotype: serosubtype: immunotype.

In addition to serological classification based on surface antigens, classification of meningococci on the basis of genetic relatedness has been found to be very useful for purposes of global epidemiology and identification of virulent clones with epidemic potential [3,4]. The principal method for doing genetic typing is multilocus enzyme electrophoresis or enzyme typing [5], but pulsed field gel electrophoresis of restricted whole-cell DNA also appears to have potential for determining genetic relatedness [6]. Using genetic typing along with serological analysis, Achtman and coworkers have extensively studied the global epidemiology of group A meningococcal disease [3]. Caugant and coworkers, using enzyme typing, identified a genetic clone called ET5 that, together with closely related strains, has been responsible for recent group B epidemics in Norway, Cuba, Chile, and Brazil [4].

B. Description of the Disease

The clinical manifestations of meningococcal disease are diverse and range from the asymptomatic carrier state to fulminant meningococcemia, which can progress very rapidly, often leading to death in 12–48 hr from the onset of symptoms. Most systemic disease, however, is manifest in the form of meningitis, meningococcemia, or both. Meningococcemia may be benign, severe, fulminant, or chronic. Associated with these primary disease states may be a variety of neurological and/or immunological complications. The clinical aspects of meningococcal disease have recently been reviewed by Cartwright [7].

In the preantiserum, preantibiotic era, the case fatality ratio for meningococcal disease was about 65–80%. Treatment with antimeningococcal antiserum, introduced in 1908 [8], eventually reduced the mortality rate to about 20–30%, and the level was further reduced to the current level of 4–15% by the discovery and use of antibiotics beginning with the use

of sulfanilamide in 1937 [9]. Even with antibiotic treatment, the prognosis for cases of fulminant meningococcemia without meningitis is quite poor. Case fatality rates varying from 15 to 71% have been reported for such cases in recent years [10].

Meningococcal disease primarily affects young children, but the age distribution varies with the serogroup [11] and the serotype or genetic clone of the infecting strain [12]. The peak incidence of endemic meningococcal disease due to all serogroups combined to about 6 months to 1 year of age, which corresponds to the age when serum antibody levels are lowest [13,14]. During epidemics, the median age of cases increases to the 5- to 10-year-old range [10,15,16].

C. Historical Disease Pattern and Geographic Distribution

Historically, meningococcal disease has occurred worldwide, often in large epidemic waves, with a periodicity of about 10 years. These periodic epidemics have been superimposed on a background of endemic disease, which is epidemiologically distinct. Endemic disease is usually much more heterogeneous with respect to both the serogroup and subcapsular antigens expressed on causative strains [10,17]. The attack rate during endemic periods is normally about 1:100,000 to 3:100,000 per year in most countries [12,14]. Epidemics, on the other hand, involve attack rates from about 10:100,000 to as high as 400:100,000 to 500:100,000 per year and most often have been caused by group A strains [10,18,19]. Serogroup B and C strains generally are most prevalent during endemic periods, but they have also been responsible for numerous outbreaks and epidemics of a reduced scale [15,16,19–22]. Epidemic serogroup A disease has largely been absent from the United States and most European countries since 1950 but is still a major problem in many areas of the world, especially the meningitis belt in Central Africa and China. Meningococci of serogroups Y and W135 cause a much smaller but not insignificant amount of disease, particularly in complement-deficient individuals [23]. Systemic disease due to serogroups 29E, H, I, K, L, X, and Z is rare.

Recently, well over half of all meningococcal disease in North and South America and Europe—including epidemics in Norway, Cuba, Brazil, and Chile—has been due to group B [15,16,20,21]. Epidemics or major outbreaks of meningococcal disease usually involve a single predominant strain or clone [3,12,15,20,24], but in prolonged epidemics the antigenic profile of the causative strain tends to become more heterogeneous with time. Certain serotype/ser-

osubtype combinations have been found to be associated with epidemic group B and group C disease. For group B disease, serotype 2 strains (mostly 2a:P1.2) were most common in the early 1960s [22,25] but were gradually replaced by 2b:P1.2 strains between the late 1960s and about 1980, depending on the country [12,22,26]. More recently, several closely related strains—including types 15:P1.16 [12,24], 4:P1.15 [20,21], and 15:P1.3 [15]—have emerged as epidemic strains. These strains have been shown by Caugant et al. [4] to belong to a cluster of genetically closely related strains called the ET5 complex. For group C disease, strains with the 2a:P1.2 and 2b:P1.2 antigenic phenotype still predominate [26,27].

D. Current Vaccines

Currently licensed meningococcal vaccines consist of different combinations of the purified high-molecular-weight CP from serogroups A, C, Y, and W-135. In addition, a group B/C outer membrane protein-C CP vaccine has been licensed in Cuba [28,29]. The group A and group C CP vaccines, which have been quite successful, were developed in the late 1960s by Gotschlich et al. [30,31]. These vaccines have been shown in multiple controlled field trials to provide excellent protective immunity in adults, but efficacy in young children varies with the age of the children and with the vaccine [32]. In an important trial in Finland, the group A vaccine was found to be effective in children as young as 6 months, but a second dose was required about 3 months after the first [33]. Gold et al. [34] concluded that a protective level of antibody against group A could be maintained throughout childhood by an appropriately timed sequence of immunizations. The group C polysaccharide, however, is less immunogenic in children under 2 years [35]. In an efficacy study of group C polysaccharide in young children in Brazil, no protection was observed in children under 24 months of age, and in children 24 to 36 months of age, the efficacy was only 52%. Although there is evidence that the vaccine used in this study may not have been of the highest quality [36], measurements of antibody responses in young children support the conclusion that the C vaccine is less immunogenic in children under 2 years of age than the A vaccine [34].

It is also important to note that children appear to acquire antibodies to the A CP by natural immunization more quickly and in greater amounts than antibodies to group C CP [34]. Thus, the apparent difference in the antibody responses of children to the A and C vaccines may simply reflect the different levels

and rate of increase of preexisting antibodies to the respective polysaccharides at the time of vaccination.

The efficacy of the Y and W-135 CP vaccines has not been proven due to the low incidence of disease caused by strains of these serogroups. They were licensed on the basis of molecular size, chemical purity, and their capacity to induce high titers of bactericidal antibodies. Since 1984 a tetravalent A, C, Y, W-135 CP vaccine has been given to all U.S. military recruits upon entrance into basic training. During that time there have been no reported cases of Y or W-135 meningococcal disease in vaccinated individuals [22]. This observation provides some evidence for efficacy of Y and W-135 vaccines.

Both the initial antibody response and the persistence of elevated titers of antibody to the CP vaccines is dependent on the quality of the vaccine used, the age of the vaccinated individuals, and the antibody level before vaccination [37]. Vaccine quality is to a large extent determined by the molecular size of the polysaccharide. High molecular weight and a high degree of aggregation of the capsular polysaccharides are associated with greater immunogenicity [38,39]. The presence of a stabilizer such as lactose and control of the cold chain are required to preserve the quality of the vaccines, particularly the group A vaccine [38]. The duration of protection, as determined by efficacy trials or by monitoring of anti-CP antibody levels, is much better in adults than in children. In a study in Air Force recruits, elevated levels of anticapsular antibodies and serum bactericidal activity against a group C strain were found to persist for at least 10 years after immunization [40]. After 10 years, 75% of the recruits had ≥ 2 $\mu g/mL$ of anti-A antibody, and 85% had ≥ 2 $\mu g/mL$ of anti-C antibody. In children, however, the persistence of anti-CP antibody and the duration of protection is less satisfactory. In a case control study in Burkina Faso [41], efficacy of the A vaccine in children less than 4 years of age decreased from 100% the first year to 8% by the third year. In children of ages 4 years and older, the efficacy decreased from 85% the first year to 67% the third year.

The A and C vaccines are generally well tolerated and have now been given to many millions of people without any fatalities or serious permanent sequelae. Nevertheless, the CP vaccines are not currently recommended for routine use in civilian populations in industrialized countries [42]. They are used primarily to control epidemics and localized outbreaks and for immunization of high-risk populations [43].

The deficiencies associated with the licensed meningococcal A and C CP vaccines cited above and the lack of an effective group B vaccine have prevented the existing CP vaccines from being used on a routine

basis in children. In addition, group A disease has historically appeared as large epidemics that occur with a periodicity of about 10 years. The relatively short duration of protection provided young children by the current group A vaccine makes it unsuitable for use for routine vaccination because protection would not likely extend to the next major epidemic.

II. CONJUGATE CAPSULAR POLYSACCHARIDE VACCINES

A. Conjugate Vaccines for Groups A and C

In the wake of the highly successful *Haemophilus influenzae* type b (Hib) conjugate protein-polysaccharide vaccines, it is widely anticipated that similar vaccines consisting of protein-polysaccharide conjugates of meningococcal A and C CP will be equally successful. The deficiencies associated with the meningococcal A and C CP vaccines can most likely be attributed to their T-cell–independent properties, but it is not entirely clear why the CP vaccines perform better in adults than in children. The maturation of the immune system has been cited, but other factors may also be important. In adults, the high-molecular-weight CPs appear to be able to stimulate CP–specific B lymphocytes and induce production of antibody by cross-linking the immunoglobulin surface receptors [44]. Most adults have likely had exposure to the CPs through colonization by meningococci or other cross-reacting organisms. If the cell associated CP behaves as a T-cell–dependent antigen, then natural priming can be considered a T-cell–dependent process. Thus, when adults are vaccinated with the meningococcal CP vaccines, they likely respond with secondary type of antibody response.

Preparation of optimized meningococcal polysaccharide–protein conjugate vaccines for serogroups A and C requires evaluation of a number of variables. These variables include which protein carrier to use, the size of the oligosaccharide or polysaccharide moiety, the conjugation chemistry, the substitution ratio, and the use of an adjuvant. Since the Hib conjugate vaccines were licensed, many clinical studies have been done to analyze and compare the characteristics of the immune response induced by each. These studies have produced a great deal of important information about the design, use, and effectiveness of conjugate vaccines that can be useful in optimizing the design of meningococcal conjugate CP vaccines. One Hib vaccine, which contained the meningococcal outer membrane protein complex (PRP-OMPC) as the carrier, behaved differently in several respects from those

that used tetanus toxoid (TT), diphtheria toxoid (DT), or the CRM_{197} nontoxic mutant toxin as the carrier. The PRP-OMPC vaccine was able to induce an antibody response in 2-month-old children after a single dose, whereas the vaccines with DT or TT as carrier required two or three doses [45,46]. A second and third dose of the PRP-OMPC vaccine, however, did not result in a substantial boost in antibody levels, and after three doses, the quantity, avidity, and bactericidal potency of the antibodies induced were lower than the antibodies induced in children by three doses of the toxoid-PRP conjugates [45]. Interestingly, an immunization schedule consisting of one dose of PRP-OMPC followed by two booster doses with a toxoid-PRP vaccine resulted in higher antibody levels at each stage in the vaccination schedule than three doses of a single-conjugate vaccine [48]. Carrier priming between 1 and 6 months of age was important for an optimal antibody response to the toxoid-based conjugates but was not required for the PRP-OMPC conjugate [49]. On the other hand, higher preexisting maternal antibody to tetanus toxoid in infants resulted in a reduced antibody response to the tetanus toxoid–PRP conjugate vaccine [50], and boosting the level of anti–tetanus toxoid antibodies in adults did not increase the anti-PRP antibody response to vaccination with PRP–tetanus toxoid vaccine [51].

The optimal length of the polysaccharide fragment to couple to the protein carrier was studied by Jennings et al. [52,53] using type III group B streptococcal polysaccharide conjugated to tetanus toxoid. They found that an intermediate length of oligosaccharide consisting of about 14 repeating units gave a greater functional antibody response in rabbits than shorter (6 repeating units) or longer (25 repeating units) fragments. They suggest that the shorter oligosaccharides lack the internal conformational length-stabilized epitope that is postulated to induce functional antibodies, and the longer fragments may result in the vaccine beginning to exhibit T-cell independence.

Several human studies have now been done with meningococcal group A and C conjugate vaccines produced by Sclavo R&D Vaccines (Siena, Italy) using the CRM_{197} mutant diphtheria toxin as the carrier [54–56]. These vaccines were produced by coupling oligosaccharides with an average chain length of 6 to the CRM_{197} protein carrier by reductive amination of the oligosaccharides followed by activation with the N-hydroxysuccinamide diester of adipic acid and reaction of the activated oligosaccharides with the CRM_{197} to give an oligosaccharide to protein ratio of about 0.25 to 0.3 [55]. The resulting vaccines, which were adsorbed to aluminum hydroxide, were tested for safety and immunogenicity in mice and rabbits and

then in human volunteers [55]. The conjugates were clearly more immunogenic in mice than the free polysaccharides. In the human study, a good antibody response to the first dose was obtained, but a second dose resulted in only a slight increase in antibody. In a further phase II study in 50 adults [54], the conjugate A and C vaccines were compared to an approved tetravalent vaccine (Menomune, Connaught Laboratories, Inc., Swiftwater, PA). The conjugates were given at three dosage levels ranging from 5.5 to 22 μg of conjugated polysaccharide, and the approved vaccine was given at the standard dosage of 50 μg of each polysaccharide per dose. The conjugate vaccines induced bactericidal antibody titers against a group C organism that were two- to threefold higher at 30 days postvaccination than titers induced by the licensed tetravalent CP vaccine. As determined by an isotype-specific enzyme-linked immunosorbent assay (ELISA), however, the conjugate vaccine induced antibody responses that were not significantly different than those of the approved polysaccharide vaccine for any of the antibody isotypes. At 1 year postvaccination, antibody levels remained highest in those volunteers who received the approved CP vaccine. This suggests that the approved CP vaccine may be better for use in adults than a conjugate of this design.

The A-plus-C conjugate vaccine was also tested for safety and immunogenicity in about 300 Gambian infants 8 to 10 weeks of age [56]. The conjugate vaccine, which contained 11 μg of each polysaccharide coupled to 49 μg CRM_{197} protein, was compared to an approved A-plus-C CP vaccine (Menpovax A + C, Biocine, Siena, Italy). Several different vaccine schedules were used, and blood samples taken were limited to two per child. The conjugate vaccine was found to be safe and immunogenic. The antibody levels induced by the conjugate vaccine as measured by ELISA after two doses at 2 and 6 months of age were about the same (group A) or double (group C) the antibody levels induced by the CP vaccine after two doses at 3 and 6 months. Three doses of conjugate vaccine at 2, 3, and 4 months resulted in titers that were twofold higher than the titers obtained after two doses. By 3 months after the third dose, the titers had fallen to about 25–30% of the peak levels. The geometric mean anti-C CP antibody titer induced by the conjugate vaccine was higher after one dose at 6 months than after two doses at 2 and 6 months. This raises the possibility that the first dose of vaccine given at 2 months induced a state of partial unresponsiveness.

Although this first conjugate A-plus-C vaccine shows improved immunogenicity in children as compared to unconjugated polysaccharide, it not clear whether the design of the vaccine is optimal. For ex-

ample, the average length of the oligosaccharides coupled to the CRM_{197} carrier (six repeating units) may have been too short. Oligosaccharides consisting of about 14 repeating units was optimal for inducing the best antibody response to a conjugate of type III group B streptococcal polysaccharide [52,53]. The results to date with the conjugate vaccines are encouraging and will hopefully lead to a product that can be used for routine vaccination of infants and young children to produce a solid, long-lasting protective antibody response.

B. Other Approaches to Improved A and C Vaccines

Other approaches to the development of an improved vaccine for groups A and C are also being successfully pursued. One such approach is the use of an anti-idiotype vaccine. Westerink et al. [57] performed sequence analysis on the variable regions of an anti-idiotype antibody that mimics meningococcal group C polysaccharide and identified the amino acid sequence that was responsible. Based on this peptide sequence, they prepared a synthetic peptide vaccine which was able to induce in mice protective antibodies specific for group C polysaccharide. To improve the immunogenicity of the peptide a lauroyl group was attached to the N terminus, and the modified peptide then complexed to proteosomes. These exciting results demonstrate the validity and feasibility of this alternative approach. Further studies should be done to evaluate the potential of this vaccine in human volunteers.

Some of the approaches being pursued in the development of a group B vaccine involve the use of subscapsular antigens, many of which are shared by meningococci of different serogroups. In the event a highly successful group B vaccine is developed, it may be equally effective against meningococci of other serogroups.

C. Prospects for a Conjugate B Vaccine

Extension of the polysaccharide-protein conjugate approach to a group B vaccine is not straightforward since the purified group B capsular polysaccharide (B CP), which is a homopolymer of $\alpha(2\rightarrow8)$-linked N-acetylneuraminic acid, is a poor immunogen [58]. An explanation for the poor antigenicity of the B CP is provided by the studies by Finne et al. [59], who demonstrated the presence of polysialic acid chains with the same structure as the B polysaccharide in glycoproteins of developing and adult tissues. The chains of polysialic acid associated with embryonic tissue are relatively long (>12 residues) and react with antibodies to B polysaccharide [60]. The polysialic acid chains become significantly shorter soon after birth, but the longer, embryonic form has been detected on certain adult tissues such as NK cells [61] and discrete areas of the brain. In addition, it appears to be transiently reexpressed in adults during muscle regeneration in several pathological situations and on certain types of tumor cells [62]. The implication of this molecular mimicry is that the use of a vaccine based on the B CP might induce autoimmunity. Although this is a legitimate concern, there have been no reports of adverse effects associated with the presence of either natural or vaccine-induced anti-B CP antibody. The immune system does not appear to recognize short, linear oligosaccharides of $\alpha(2\rightarrow8)$-linked polysialic acid as foreign, but can recognize and respond, to a limited extent, to larger conformational epitopes [63–67].

Two approaches have been used to attempt to increase the immunogenicity of the B CP. The first attempts were based on observations that B CP associated with whole cells or complexed to OMPs had greater antigenicity and immunogenicity than free CP. Candidate vaccines consisting of noncovalent complexes of B CP and OMPs were prepared and evaluated in phase I and phase II human safety and immunogenicity studies [68,69]. These studies demonstrated that the vaccines were safe and that the B CP was moderately immunogenic when bound to the OMPs. The optimal antibody response to the B CP was obtained with a 1:3 w/w ratio of CP to protein [69]. Lifely and coworkers optimized the preparation of noncovalent complexes to reduce the amount of unbound B CP and adsorbed the complexes to aluminum hydroxide. In a human safety and immunogenicity study [70] they demonstrated that (1) antibody responses could be induced that persisted for at least a year after vaccination at geometric mean levels two to three times the prevaccination level; (2) the antibodies induced could provide passive protection in a iron dextran–sensitized mouse model; (3) a booster effect could be obtained after a second dose; and (4) 93% seroconversion (>twofold increase) could be obtained after three doses.

The mechanism by which the complexing of the B CP with the OMPs enhances its immunogenicity is not fully understood. Binding of the B CP to the OMP may stabilize a conformational epitope [66,67] that is characteristic of cell-associated polysaccharide and important for immunogenicity. In addition, an adjuvant effect associated with the OMP is suggested by results obtained with meningococcal outer membrane proteins used as proteosomes to improve the immunogenicity

of hydrophobically bound antigens such as synthetic peptides, lipopolysaccharides, and capsular polysaccharides [71–73]. The OMPs have been shown to have mitogenic activity [74] and to induce B lymphocyte costimulatory factor B7-2 [75]. It is unclear whether noncovalent complexing of the B-CP to the OMP converts it to a T-cell–dependent antigen.

The antibodies to B CP induced by natural infections or vaccination with noncovalent complexes are predominantly of the IgM class [63,68,70], have lower avidity at 37°C, than at 4°C, and are generally not functional in a bactericidal assay with complement from the same species [63,64]. These characteristics are not shared by the antibodies to group C CP. On the other hand, anti-B CP antibodies have been shown to support opsonophagocytosis with homologous complement and to protect in animal models [76,77]. At higher antibody concentrations, protection could also occur by anti B CP antibodies binding to the organism and neutralizing the anticomplement activity of the sialic acid capsule [78,79]. Lack of bactericidal activity with homologous complement, however, causes significant doubt to remain about whether these anti-B CP antibodies are protective against group B disease in humans.

The second approach to improving the immunogenicity of the B CP has been to prepare covalent conjugates with appropriate protein carriers. By this approach the B CP can be converted to a T-cell–dependent antibody and probably stabilize conformational epitopes as well. Several different methods have been used in the preparation of B CP–protein conjugates. Jennings and Lugowski [80] coupled meningococcal A, B, and C CP to tetanus toxoid by reductive amination and found that their approach was effective for group A and group C polysaccharides but not for B CP. The anti–B CP antibody response obtained in animals was directed primarily against the linkage point between the CP and the protein. In subsequent studies, Jennings and coworkers [81] attempted to overcome the poor immunogenicity of the B CP by specific chemical modification. The N-acetyl groups were removed from the CP and replaced by N-propionyl groups. This modified structure was subsequently covalently linked to tetanus toxoid. Vaccination of mice with this conjugate resulted in high levels of IgG antibody cross-reactive with the group B CP. Several specificities of antibodies were identified in the antisera. One population reacted with the modified polysaccharide but not with the native B CP. Another population of antibodies reacted with purified group B CP, and a third population reacted with whole cells or B CP linked to an affinity column via a long spacer arm but not to soluble B CP. Only the third

antibody population that mostly consisted of IgG_{2a} and IgG_{2b} isotypes was found to be bactericidal for group B meningococci. These antibodies appeared to be reacting with an epitope on the cell-associated CP that was not present on free, soluble B CP [82].

A different conjugation methodology was used by Devi et al. [83] and Bartoloni et al. [84], who linked unmodified, high-molecular-weight CPs to tetanus toxoid or CRM_{197} through an adipic acid spacer arm. Devi prepared tetanus toxoid conjugates of CP from group B and group C N. meningitidis, E. coli K1, and E. coli K92, which is an alternating copolymer of $\alpha(2\rightarrow8)$ and $\alpha(2\rightarrow9)$–linked N-acetyl neuraminic acid. These conjugates were injected as saline suspensions in mice and were found by ELISA to induce antibody responses consistent with a T-cell–dependent antigen. The E. coli K92 CP conjugates induced a good antibody response against both B CP and C CP. Both IgM and IgG antibodies were induced in each case, but the bactericidal activity of the antibodies was not determined. The anti–B CP antibodies showed lower binding at 37°C than at 22°C, suggesting that the quality of the antibodies was similar to that of antibodies induced by noncovalent complexes or natural infections. Using similar methods, Bartoloni et al. [84] prepared tetanus toxoid and CRM_{197} conjugates of native B CP and studied the immunogenicty of the vaccines in mice and the specificity of the antibodies induced. Both IgG and IgM antibodies specific for B CP were induced and found to be bactericidal, but most of the antibodies induced by the vaccine were directed against the linkage region of the conjugate.

Although conjugation of B CP or chemically modified B CP to a carrier protein enhances immunogenicity and converts the polysaccharides to T-cell–dependent antigens, it is not clear whether the quality of the antibodies induced is adequate to provide solid, long-term protection. Although both IgG and IgM antibodies are induced in animals and bactericidal activity can be demonstrated, the antibodies show decreased avidity at 37°C and have not been shown to support bactericidal activity with a homologous complement source. A study of antibodies induced by several conjugate B CP vaccines in rhesus monkeys failed to demonstrate bactericidal activity with homologous complement [85]. Although strong correlation has been established between the presence of serum bactericidal activity and immunity to meningococcal disease [86], it has been suggested that phagocytosis may play a greater role in protection against group B disease than in protection against meningococcal disease caused by the other serogroups [87]. It is of interest, however, that studies with human IgM paraproteins from individuals with monoclonal gam-

mopathy have shown that some of these antibodies with specificity for B CP have bactericidal activity with human complement providing antibody concentrations are sufficiently high [88].

The question of the safety of antibodies induced by conjugate B CP vaccines is also still unresolved. Studies of the binding of a variety of monoclonal and polyclonal antibodies with specificity for B CP or chemically modified B CP showed that all the antibodies that bound to high-molecular-weight $\alpha2{\rightarrow}8$–linked polysialic acid (colominic acid) could also bind to the polysialic acid present on glycopeptides of human embryonic brain [60]. One polyclonal mouse serum made against N-propionyl B CP using aluminum hydroxide as an adjuvant did not bind to colominic acid or to polysialyl glycopeptides but had bactericidal activity against group B meningococci. If this result is not simply due to the low sensitivity of the binding assay, it suggests that there may be potential for using the N-propionylated B CP conjugates as a vaccine to induce antibodies that are bactericidal against group B meningococci but do not cross-react with polysialic acid on human tissues.

Results of human studies of N-propionyl B CP conjugate vaccines may soon be available and will provide important information about the potential of these vaccines to induce protective immunity against group B disease in humans.

III. SUBCAPSULAR VACCINE APPROACHES FOR GROUP B

A. Existing Outer Membrane Protein Vaccines

The candidate group B vaccines that have been most extensively tested in humans are all based primarily on a complex mixture of OMPs extracted directly from cells or from outer membrane vesicles using either sodium deoxycholate or the zwitterionic detergent Empigen BB. Isolation of the OMPs using deoxycholate is effective in removing most of the LPS and phospholipid but a residual of 5 to 7% LPS remains associated with the proteins [89] The OMPs extracted in this way assume a membrane vesicle–like morphology and presumably retain a relatively native conformation [89]. Use of Empigen BB for protein purification can yield OMPs with less than 1% associated LPS but results in somewhat greater dissociation of the membrane structure, resulting in a range of subunit or particle sizes from vesicles to multimeric complexes of about 300 kDa [90].

The capacity of vaccines based on the outer membrane proteins to induce protective immunity

against group B meningococcal disease has now been demonstrated in several large field trials and case-control studies [28,29,89,91]. A summary of the results of these trials is given in Table 1. Although efficacy in the range of 51–83% was demonstrated in each of the trials, improvements are clearly needed. Each of these efficacy trials has contributed important information to our understanding of human immunity to group B N. meningitidis and to the knowledge base required for development of more effective group B vaccines. In several of the trials [90,91] there was an indication that efficacy was higher in the first 6 to 10 months of the study, which suggests that the duration of the antibody response needs to be increased. The trials in Chile and Brazil that included young children showed poor protection in children under 4 years of age. The reason for this is not clear, but it is consistent with a rather poor bactericidal antibody response in this age group. The capacity of young children to mount a good overall antibody response to the outer membrane proteins is seen by the results of ELISA assays on pre- and postvaccination sera of children in the studies in Brazil and Chile [90,92]. The immunogenicity of the Norwegian vaccine in young children has not been reported. In the Chilean trial, the 1–4-year-old age group had higher geometric mean antibody responses by ELISA than the older children. These results suggest that the specificity and/or the isotype of most of the antibodies induced in young children were not optimal for expression of bactericidal activity.

A further observation of importance was the apparent lack of serotype or serosubtype specificity of the protection observed in the trials in Norway and Brazil, where there was significant heterogeneity in the serosubtype and/or serotype of the case isolates [28,29,93]. This result suggests that although PorA is immunodominant in some animals and appeared to induce bactericidal antibodies in at least some of the volunteers in these trials [94,95], protection was likely due to antibodies against multiple antigens. OMP vaccines of the kind tested in field trials to date contain multiple proteins that can potentially induce bactericidal antibodies, and in some cases a significant amount of LPS was also present. Some of these OMPs remain relatively uncharacterized, which makes analysis of the specificity of the bactericidal antibodies induced by these vaccines complex. Analysis of the bactericidal antibody responses in the Norwegian trial provided evidence that both PorA and Opc induced a significant amount of bactericidal antibody [94,96]. Additional studies are needed to identify other OMPs that are important for inducing protective antibodies. The role of the residual LPS present in several of the

Table 1 Recent Meningococcal Group B Vaccine Efficacy Trials

Years	Place (Ref.)	Trial type[a]	Vaccine[b]	OMP type and serosubtype	Volunteer ages	Estimated efficacy	Duration of study
1987–89	Cuba [28]	RDBC	OMV+CP+HMWC+Al(OH)$_3$ (VA-MENGO BC)	4:P1.15	10–14 yr	83%	16 mo
1987–89	Chile [90]	RDBC	OMP+CP+Al(OH)$_3$	15:P1.13	1–21 yr 5–21 yr[c] 1–4 yr[c]	51% 70% NP[d]	20 mo
1988–91	Norway [91]	RDBC	OMV + Al(OH)$_3$	15:P1.16	14–16 yr	57%	17–29 mo
1990–91	São Paulo, Brazil [29]	CC	VA-MENGO BC	4:P1.15	3 mo–7 yr ≥48 mo[c] 24–47 mo[c] 3–24 mo[c]	54% 74% 47% NP[d]	12 mo
1990–91	Rio de Janeiro, Brazil [98]	CC	VA-MENGO BC	4:P1.15	6 mo–>9 yr 48 mo[c] <48 mo[c]	54.1% 71% 28%	12 mo

[a]RDBC = randomized, double blind, controlled efficacy trial; CC = case-control efficacy study.
[b]OMV = deoxycholate extracted outer membrane vesicles, HMWC = high molecular weight complex of outer membrane proteins; OMP = Empigen BB purified outer membrane proteins fractionated by gel filtration to remove the lower molecular weight proteins including the Opa, Opc, and H8 proteins.
[c]Subset of complete study group.
[d]NP = no protection.

vaccines also needs further clarification. The presence of LPS could affect vaccine immunogenicity in several ways. It might directly induce protective antibodies or endotoxin-neutralizing antibodies, it might be important in stabilizing native OMP structure, and it might act as an adjuvant. Without a better understanding of the important protective antigens in these complex OMP vaccines, it will be difficult to establish adequate quality control for their production.

The efficacy results observed in the field trials of group B OMP vaccines discussed above may have been modulated by the effects of prior or subsequent carriage of meningococci or other bacteria with cross-reactive antigens. In the Chilean trial, evidence was obtained that vaccination resulted in an enhanced antibody response to subsequent natural infections [90,97]. In the case-control study in Rio de Janeiro, Brazil [98], a higher vaccine efficacy was observed in areas of highest disease incidence. This may reflect a priming of the population by carriage of the epidemic strain or an enhancing effect of postvaccination carriage on the level of vaccine-induced efficacy.

B. Improved Outer Membrane Protein Vaccines

Several attractive options are being pursued by various investigators to develop improved OMP vaccines. These approaches include (1) genetic engineering of custom vaccine strains that express desirable antigens, in some cases mutltiple copies, and do not express undesirable antigens [99–101]; (2) growth of the vaccine strain under iron-limiting conditions to induce expression of the iron-uptake proteins [102]; (3) combination of the OMPs with detoxified LPS and/or liposomes to attempt to reconstitute the outer membrane and present the OMPs in a more native configuration [103,104]; and (4) presentation of the OMPs and LPS in their native state (native outer membrane vesicles) as a mucosal vaccine [105].

Starting with a spontaneous mutant that was PorB deficient, Van der Ley et al. constructed multivalent vaccine strains expressing three antigenically different PorA variants at the same time. The new porA genes were inserted in the place of one of the opa genes and the rmpM gene [99]. Additional genetic alterations were made to delete the porB gene and the cps gene which disabled expression of PorB, capsular polysaccharide, and the lacto-N-neotetraose group on the LPS. The latter two components both share the same structure as oligosaccharides in certain human tissues. Poolman and coworkers used two such trivalent PorA vaccine strains to prepare a hexavalent vaccine that contained 6 different PorA proteins [100]. This vaccine, which was modeled after the Norwegian vaccine,

consisted of deoxycholate-extracted vesicles from each of the two vaccine strains. Clinical studies with this vaccine are under way [106] but results are not yet available.

A second approach used by Van der Ley et al. to express multiple serosubtype specific epitopes in a single strain was to insert foreign PorA VR1 (loop 1) or VR2 (loop 4) peptide sequences into loops 5 and 6 of PorA, thereby elongating these loops with additional serosubtype specific epitopes [107]. When injected into mice as components of outer membrane complexes, these hybrid PorA proteins were effective in inducing bactericidal antibodies against the foreign serosubtype specific epitopes as well as the original epitopes. This approach offers the possibility of further increasing the number of serosubtype epitopes presented in a vaccine while minimizing the number of different vaccine strains required and also minimizing the amount of residual endotoxin contained in the vaccine.

The importance of deleting the class 4 OMP gene (rmpM) from vaccine strains to be used for production of OMP vaccines is not clear. Under certain conditions antibodies to the class 4 OMP have been shown to block bactericidal activity of other antibodies [108]. The OMP vaccines used in the efficacy trials discussed above all contained this protein, but no evidence has been reported to indicate that its presence adversely affected the efficacy of the vaccines. To address the question of whether the presence of the class 4 OMP in a vaccine has a negative impact on vaccine effectiveness, two vaccines for human use were prepared from a class 4 OMP deletion mutant [101] and its isogenic parent. These two outer membrane vesicle vaccines were tested in a Phase I clinical study to compare their capacity to induce bactericidal antibodies. The results of the study showed no significant difference in the geometric mean bactericidal titers induced by the two vaccines [109]. These results suggest that for this type of vaccine the presence of RmpM does not have a significant adverse effect the bactericidal titers induced by the vaccine.

OMP vaccines might also be improved by including several additional antigens that have emerged as possible vaccine candidates. The OMP vaccines tested in efficacy trials did not contain the iron-regulated proteins (IRP) [90,110] and could possibly be improved by including them (see discussion of Tbp2 below). One approach to including these proteins is to simply grow the vaccine strain under iron limiting conditions [102]. It is not clear, however, if this approach will be the most effective way to present the IRP. Another antigen of potential importance in OMP vaccines is Opc (see discussion below), an opacity protein that

appears to function as an invasin [111]. Although nearly all strains appear to have the capacity to express Opc its expression is subject to phase variation at a relatively high frequency. The Norwegian vaccine contained Opc, but only a minor percentage (~20%) of the case isolates obtained during the Norwegian trial expressed it [96]. Genetic alteration of vaccine strains to stabilize high-level expression of Opc might be useful.

It may be possible to improve the quality of the antibody response to OMP vaccines by presenting them in a way that more closely mimics their natural environment in the outer membrane. Two approaches have been taken in an effort to reconstitute the OMPs into an outer membrane–like environment. Wetzler et al. [112] found that when gonococcal OMPs were combined with liposomes, a higher percentage of the antibodies induced in rabbits could bind to intact organisms. The binding of the OMPs to liposomal lipids may stabilize the conformation of the OMPs and/or mask immunogenic portions of the proteins that are normally buried in the lipid bilayer of the outer membrane. Noncovalent complexing of OMPs to alkaline detoxified meningococcal LPS has also been used in our laboratory [103,113] and by others [114] in an attempt to partially reconstitute the outer membrane environment and add the LPS to the vaccine as an antigen.

Even without the improvements discussed above, some increase in the effectiveness of existing OMP vaccines appears to be possible by giving a third dose of vaccine 6 months or more after the second dose. Studies with both the vaccine developed in Cuba and the vaccine developed in Norway showed a substantial boost in antibody titers after a third dose of vaccine [28,115,116].

C. Vaccines Based on Specific Outer Membrane Proteins

The current OMP vaccines contain most or all of the proteins of the outer membrane and are, therefore, fairly complex vaccines that present some challenges from the point of view of quality control and standardization. From this perspective the development of vaccines based on a single well-characterized OMP is an attractive alternative. Significant progress has been made toward the development of several vaccines of this type. Among the more promising candidates are vaccines based on PorA; synthetic peptides derived from PorA; transferrin binding protein 2; and Opc.

Multivalent PorA Vaccines

PorA, which bears the determinants of serosubtype specificity, has a number of characteristics that recommend it as a vaccine candidate. Among these are its capacity to induce bactericidal antibodies that are protective in animal models [117], its relatively stable expression, and its moderate degree of antigenic variation [2,118]. The structural gene was initially cloned and sequenced by Barlow et al. [119]. It has subsequently been amplified from many different serosubtypes using the polymerase chain reaction, and the entire gene, or the variable regions, were sequenced [120,121]. Based on comparative DNA sequences of PorA from a number of different serosubtypes two principal variable regions, VR1 and VR2, have been identified. When the PorA sequence was fit into the beta barrel model of bacterial porins and epitope mapping done with serosubtype specific monoclonal antibodies, it was found that the principal serosubtype epitopes corresponded to VR1 and VR2 which were located near the tips of the surface exposed loops 1 and 4 [122,123]. Thus each PorA carries two different serosubtype specific epitopes, which appear to serve as a targets for bactericidal antibodies.

In an effort to develop a vaccine based on purified PorA, a system for the production of large amounts of pure, endotoxin-free PorA was developed by expressing the cloned gene as inclusion bodies in the Gram positive bacterium *Bacillus subtilis*. PorA could be quite easily isolated and purified, but solubilization of the inclusion bodies required denaturing conditions. The resultant purified PorA was found to be able to induce a good, relatively cross-reactive, antibody response in animals, but the antibodies were devoid of bactericidal activity. It was discovered, however, that complexing the denatured PorA with lipopolysaccharide from homologous or heterologous species enabled the PorA to renature and acquire the capacity to induce bactericidal antibodies in animals [124]. Further work showed that when the denatured PorA was combined into liposomes, the same renaturing effect could be achieved in the absence of lipopolysaccharide [125]. Liposome-based vaccine preparations containing PorA were found to induce serosubtype-specific antibodies that were bactericidal and protective in the infant rat meningitis model [104]. These results provide evidence that a purified PorA vaccine may be feasible. Although cross-reactive antibodies were induced by the denatured form of the protein, the antibodies with bactericidal activity that were induced by LPS or liposome renatured PorA were highly serosubtype specific [104]. This means that a vaccine designed to protect against group B meningococcal disease must be

multivalent and contain all or most of the different serosubtypes (about 12 to 15) that have been identified. Even a single amino acid substitution in the peptide sequence forming a serosubtype specific epitope can lead to failure of bactericidal antibodies to recognize it [126]. This may be a problem for a vaccine of this design depending on the frequency with which mutations occur.

Two different groups have demonstrated the feasibility of producing synthetic peptides based on the PorA variable regions that are able to induce bactericidal antibodies in mice [127–129]. Both circularized, relatively long peptides and shorter peptides combined with universal T-cell epitopes in the multiple antigen peptide format have been successfully used. This raises the possibility that an effective semisynthetic and highly defined multivalent vaccine for group B could be produced. It may be possible to increase the immunogenicity of such peptides by attaching a lipid moiety at one end and combining the peptides with liposomes or proteosomes. The synthetic peptide approach would be expected to induce highly serosubtype-specific antibodies unless conserved epitopes can be identified that are able to induce protective antibodies.

Transferrin-Binding Protein 2

The proteins of the meningococcal iron uptake system have been the focus of considerable interest and research over the past 5 to 10 years. Much progress has been made in understanding both the mechanisms by which the organism scavenges essential iron from the environment and the potential of the proteins involved in this process for use as a vaccine against group B disease. The various proteins involved in the acquisition of iron are induced under conditions of low iron availability and are usually referred to as iron regulated proteins (IRPs). A number of different proteins are involved in the acquisition of iron including specific surface receptors for transferrin [130], lactoferrin [131], and heme [132], which appear to be the major sources of iron in vivo. Antibodies to an appropriate IRP might protect against disease by initiating complement-mediated lysis of meningococci and./or by binding in such way that the uptake of iron is blocked and growth stopped. Antibodies with both of these activities are induced by the transferrin receptor [133]. The transferrin receptor has been shown consist of a complex made up of two separate proteins called transferrin binding proteins 1 and 2 [Tbp1 and Tbp2]. Tbp1 has a molecular weight of about 95–98 kDa, whereas Tbp2, which is a lipoprotein, has a molecular weight in the range of 68 to 85 kDa, depending

on the strain [134,135]. Within the transferrin binding complex, Tbp2 appears to be the most active in inducing antibody that is active in bactericidal assays and in blocking the binding of human transferrin [136].

The degree of antigenic variability in Tbp2 is an important consideration in evaluating its potential as a vaccine or vaccine component. Rokbi et al. found that most meningococci could be divided into two groups or families based on the immunological and genomic characteristics of their Tbp2 molecules [137]. Group I Tbp2 have molecular weights in the range of 78–85 kDa, and group II Tbp2 have molecular weights in the range of 67–73 kDa [134,138]. Amino acid sequence homology of 76.6–81.2% was observed for different Tbp2 from the same group [139], and 47% homology was found between Tbp2 from different groups [140]. Of particular importance in assessing antigenic variability is the degree of cross-reactivity associated with functional human antibody that can block binding of transferrin or kill meningococci in the presence of complement. Gorringe et al. [141] recently demonstrated broadly cross-reacting anti-Tbp1 and Tbp2 antibodies in human sera from cases and carriers using ELISA and western blotting, but functional antibody was not measured. Ferrieros et al. [142] also found by western blotting that anti-Tbp2 antibody in three human case sera showed intragroup cross-reactivity. The cross-reactivity of bactericidal antibodies in rabbit hyperimmune anti-Tbp serum was measured by Danve et al. [133] using 11 different strains of meningococci. The serum killed about half of the strains at about the same titer but showed no activity against the other strains. These results suggest intragroup cross-reactivity of the rabbit bactericidal antibodies, but the molecular weight category of the Tbp2 produced by these strains was not given. Protection by mouse anti-Tbp in active and passive mouse protection studies was also demonstrated. In further studies, Lissolo et al. [136] demonstrated that the functional activity associated with mouse and rabbit anti-Tbp antiserum was predominantly associated with antibodies to Tbp2 rather than Tbp1. Although more basic work needs to be done to evaluate the cross-reactivity of functional antibody, Tbp2 appears to be emerging as a viable vaccine candidate. A suitable vaccine would need to contain a minimum of two different Tbp2s, one from each group. Until now no vaccines based entirely on transferrin binding proteins have been reported, but the tbp genes have been cloned and, Tbp2 expressed as a complete lipoprotein in E. coli [143,144]. The preparation and evaluation of a vaccine based on recombinant Tbp2 might be expected in the not too distant future.

Opa and Opc Proteins

The Opa and Opc proteins, previously referred to as class 5 proteins, are surface-exposed proteins that are associated with colony opacity under appropriate conditions. These proteins have a basic PI and monomer molecular weights in the range of 25 to 30 kDa [145]. Although Opc and the Opa proteins share a number of characteristics, they differ in other important respects. Opc was initially identified and characterized in group A strains by Achtman et al. [145] as a class 5 OMP that was called 5C or 5c, depending on whether it was expressed at a high or low level. After the gene for Opc was cloned and sequenced [146] it became clear that Opc was significantly different than the other class 5 proteins (Opa proteins) that had been characterized. Opc was found to have only about 27% amino acid sequence homology with Opa proteins, and although it exhibits a high level of phase variation in expression, like the Opa proteins, the mechanism involved is different [146]. Expression of Opa proteins is controlled by a series of CTCTT pentameric repeats in the DNA coding for the signal peptide. Addition or deletion of one or more pentameric repeats shifts the reading frame and in most cases results in a truncated protein. Expression of Opc, on the other hand, is controlled at the level of transcription by a string of contiguous C residues in the promoter sequence [146]. Changes in the number of C residues in the string results in changes in the efficiency of transcription. Opc further differs from the Opa proteins in that it is coded for by a single gene and it does not seem to show antigenic variation. The Opa proteins are coded for by a family of four genes that share a conserved framework interspersed by a semivariable region and two hypervariable regions [147]. Both the Opa proteins and Opc have been shown to be able to induce bactericidal antibodies in humans [148–150]. The fact that Opc is antigenically conserved makes it a somewhat more attractive candidate for use in a vaccine than the Opa proteins.

Certain Opa proteins have been shown to be effective in mediating attachment of meningococci to human epithelial cells, but Opc appears to be quite effective in mediating attachment to and invasion of both epithelial and endothelial cells [151,152]. This activity is seen only when unencapsulated meningococci with nonsialylated LPS are used. These observations suggest that Opc may play an important role in the initial stages of pathogenesis by unencapsulated meningococci colonizing the nasopharyngeal mucosal surfaces. Achtman et al. [153] and Rosenqvist [148] found that a higher percentage of throat isolates expressed a high level of Opc than did case isolates. These results suggest that Opc may be predominantly expressed and functional while the meningococci are at the mucosal surface and then turned off before they enter the bloodstream and become subject to lysis by antibody and complement.

Opc was present in the outer membrane vesicle vaccine produced in Norway and tested in the efficacy trial done there. Rosenqvist et al. [148] showed that the Opc component of the vaccine was highly immunogenic in humans and was responsible for a substantial proportion of the bactericidal antibody that was induced by the vaccine. This was especially true when the postvaccination sera were tested against strains that had heterologous serotype and serosubtype. It is not known whether these bactericidal antibodies were important in the protection observed in the trial, since the bactericidal antibodies to Opc were only effective against strains expressing high levels of Opc, and a relatively small percentage of case isolates obtained during the trial were found to have a high level of Opc expression [148]. Though strongly bactericidal, anti-Opc antibody may turn out to be most useful in preventing infection when present at the mucosal surface where it could interfere with attachment and invasion [152]. From this point of view, Opc might be an effective component of a vaccine designed to be delivered via the intranasal route.

D. Vaccines Based on LPS

The LPS of *N. meningitidis* consists of a lipid A moiety linked to one of a series of short, branched oligosaccharides of about 7 to 10 sugar residues, and is often referred to as lipooligosaccharide. A given strain may simultaneously express several different LPS structures or immunotypes which can be visualized as distinct bands on silver-stained SDS polyacrylamide gels [153–155]. The most prevalent immunotype is endogenously sialylated, which results in a terminal pentasaccharide that is largely nonimmunogenic and identical in structure to oligosaccharides present on certain human cells [158].

The use of the LPS as a vaccine or vaccine component has been hampered by several factors, including its toxicity and the molecular mimicry associated with the lacto-N-neotetraose group. Nevertheless, the LPS appears to have some potential for use as a group B vaccine or vaccine component. Anti-LPS murine monoclonal antibodies were shown by Saukkonen et al. [117] to be bactericidal and to give protection in a infant mouse model. Rhesus monkeys vaccinated with a conjugate meningococcal OMP-B polysaccharide vaccine developed high titers of anti-LPS bactericidal antibodies against the small amount of residual L3,7,9

LPS present in the vaccine. These antibodies were specifically inhibited by L3,7,9 LPS [85]. Griffiss et al. [159] were able to inhibit the bactericidal activity of antibodies in convalescent sera from children and infants with L1,8 LPS, and we have observed that a high percentage of naturally acquired bactericidal antibodies in the sera of Chilean children could be specifically inhibited with L3,7,9 LPS (unpublished data). However, in spite of these indications that LPS is capable of inducing bactericidal antibodies in humans, the antibodies to LPS induced in volunteers by LPS-containing OMP vesicle vaccines were apparently not bactericidal [94,148].

The use of LPS as a vaccine or major vaccine component has thus far met with limited success, especially with respect to the L3,7,9 immunotype. Nontoxic LPS-based vaccines have been produced in several laboratories by conjugating the oligosaccharide portion of the LPS to a suitable protein. The initial studies by Jennings and coworkers [160] used dephosphorylated oligosaccharides coupled to tetanus toxoid by reductive amination. These conjugates induced bactericidal antibodies in rabbits, but in the case of the L3,7,9 immunotype, immunogenicity was marginal. Verheul et al. reasoned that the poor immunogenicity of the L3,7,9 conjugate was due to the removal of the phosphoethanolamine groups and prepared conjugates using alternative chemical methods that preserved the phosphoethanolamine groups on the L3,7,9 and L2 oligosaccharides [161]. Although conjugates prepared in this way using tetanus toxoid or meningococcal OMP as the carrier protein induced high titers of IgG in rabbits and mice the antibodies were not bactericidal [162]. Gu and Tsai used similar chemistry to prepare a conjugate of the L8 oligosaccharide with tetanus toxoid and found it was able to induce bactericidal antibodies in rabbits [163]. Some cross-reactivity of the rabbit antibodies with L3,7,9 LPS was demonstrated by ELISA. Alternative approaches to producing a safe LPS vaccine are to detoxify the LPS by alkaline deacylation of the lipid A or decrease its toxicity by incorporating it in liposomes. Alkaline detoxified LPS binds to purified OMP hydrophobically to form soluble noncovalent complexes which can be used safely as a vaccine. We have tested one such vaccine containing L3,7,9 detoxified LPS and OMP from two different strains, 44/76(15: P1.7,16) and 8047(2b:P1.2), in a Phase I study and found that it was safe and immunogenic. Most of the bactericidal activity induced by the vaccine appeared to be directed against the OMP, and although a geometric mean threefold increase in anti-LPS antibodies was measured by ELISA, we were not able to demonstrate bactericidal activity for these antibodies.

[103]. Incorporation of this type of vaccine into liposomes may improve the results. Petrov et al. [164] found that native LPS incorporated into liposomes had greatly reduced toxicity and that the liposomal LPS was safe and moderately immunogenic in animals. They suggested the addition of a T-cell–dependent antigen to the liposomes to improve the anti-LPS response. Liposomes containing lipid A have been shown to be an effective adjuvant for presentation of a malaria antigen R32NS1 in humans [165]. In a similar manner, liposomes containing native meningococcal LPS may be an effective means of presenting one or more meningococcal OMPs to the immune system. Alternatively, it may be possible to safely give native LPS as an intranasal vaccine as a component of native outer membrane vesicles or incorporated in liposomes.

Concern about the safety of using L3,7,9 LPS containing the lacto-N-neotetraose group in a vaccine has led several investigators to attempt to identify cross reactive LPS epitopes that do not contain this group, but are capable of inducing a protective immune response against strains carrying the L3,7,9 LPS. Studies that measured the ability of immune human sera to inhibit the binding of LPS-specific monoclonal antibodies to purified LPS led to the identification of an epitope associated with a 3.6-kDa LPS that bound a bactericidal monoclonal antibody D6A, and this binding was inhibited by immune human sera [166]. Further characterization of this epitope is needed. Other investigators have attempted to define the minimal oligosaccharide structures necessary to induce an immune response to meningococcal LPS by direct chemical synthesis of the basic core structures of the LPS [167]. This elegant approach has had some success, but thus far the structures produced have failed to induce bactericidal or protective antibodies.

It is not known whether vaccination with the L3,7,9 LPS represents a significant risk. OMP vesicle vaccines containing significant amounts of L3,7,9 LPS have been given to millions of people to date without any reports of problems associated with an autoimmune response induced by the lacto-N-neotetraose group of the LPS. Also, patients recovering from systemic meningococcal disease have no known sequelae that have been reported to be associated with antibodies to the lacto-N-neotetraose group. Nevertheless the molecular mimicry that is evident demands that caution be used in pursuing human studies of vaccines based on LPS that contains the lacto-N-neotetraose group. In some cases vaccine strains have been genetically engineered to express an LPS that does not contain the lacto-N-neotetraose group [99,100]. Including LPS that consists only of the common inner core region of the oligosaccharide may not result in

induction of bactericidal antibodies cross-reactive with the L3,7,9 LPS but could induce endotoxin neutralizing antibodies.

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